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(54) Title: **PROPHYLACTIC DIETARY SUPPLEMENT BASED ON MILK**

(57) Abstract: Milk is commonly and extensively consumed in many societies where the risk and incidence of diabetes, vascular disease (CHD, CVA, PVD) and some cancers are also high. Death is a frequent sequel of systemic vascular wall damage, resulting from exposure to high sugar levels in diabetes and also from high plasma homocyst(e)ine (tHcy) levels that affect much of the population and comprise a major risk factor for vascular disease. Diabetes is similarly widespread. Given (1) widespread and regular consumption of milk, (2) the possibility to control tHcy by treating the underlying folate (and other vitamin) insufficiency, (3) the opportunity to simply include control of neural tube defects and (4) the presumed causal link between diabetes and type A1 with type B casein consumption, the invention offers remediation by supplying a population with a modified milk or milk product including (1) fortification using cobalamin, pyridoxine, folic acid, and betaine, with (2) a substantially type A2 casein fraction only. In addition, exploitation of the immunological properties of beta-casomorphin 9 (a peptide digest fraction of A2 beta-casein) may assist in control of diabetes. Practical and convenient fortified diets include treated, selected milks and food products including derivatives of milk, also selected milks together with treated cereals.

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TITLE Prophylactic dietary supplement based on milk.**FIELD**

The present invention relates to the development and use of modified forms of dairy products for use in food, the variations being intended to reduce the incidence of cardio- and cerebro-vascular disease, and also diabetes, in a population. The modifications may be imposed at the animal phase of production, and/or during a manufacturing phase.

DEFINITIONS

Vascular disease (VaD) as used herein refers to coronary (or ischaemic) heart disease (CHD), cerebrovascular disease (CVA), and peripheral vascular disease (PVD). Atherosclerosis is one associated syndrome.

Diabetes includes juvenile / IDDM/ type I, and maturity onset / type II diabetes.

Other diseases include: prematurity, loss of memory in the elderly, Alzheimer's disease, teratogenic effects; neural tube defects (NTD) such as closure problems resulting in spina bifida and the like, asthma, and cancers such as bowel (colorectal) cancer, cervical cancer and/or endometrial cell dysfunction, multiple myelomas (see Fig 2) and abnormalities in haematopoiesis.

tHcy is an abbreviation for plasma homocyst(e)ine concentration.

Milk products as used herein refers to edible foodstuffs made from milk or fractions of milk and includes for example a variety of foods containing caseins, chocolate, and the more obvious examples such as ice cream, yoghurt, and cheese, condensed milk, dried milk powder, or other milk products, even "non-dairy creamers", chocolate, cheese, and others. It also includes various forms of liquid milk such as homogenised, low-fat, high-calcium, flavoured, and other milks.

"Substantially," as used within this specification, also includes relative degrees of separation.

Chemical species, CAS numbers, and synonyms (Group I is our name for this set.)

- Betaine (trimethylglycine, CAS number 107-43-7*)
- Cobalamin (Vitamin B12, CAS 68-19-9)
- Folic acid (vitamin M, CAS 59-30-3) (Folates).
- Pyridoxine (Vitamin B6, CAS 58-56-0*) (* is the CAS number for the hydrochloride; given by way of example; not an implication that the HCl salt is specifically required).

BACKGROUND

30 Milk is commonly and extensively consumed in many societies where the risk and incidence of both diabetes and VaD is high. There is growing evidence in the form of repeatedly confirmed strong correlations that existing forms of milk of bovine origin, are linked to both groups of diseases.

Is it the proteins or the fats within dairy products that are responsible? There is good evidence
35 that human plasma homocyst(e)ine (tHcy) levels are more highly correlated with heart disease and VaD in general, and are a more accurate risk indicator than are elevated cholesterol levels (Pietrzik 1998). Graham et al (1997) provides a large survey that evaluates tHcy as an independent risk factor for VaD and furthermore, it is multiplicative with others (hypertension, smoking, etc) Vascular wall health is also seriously compromised in patients having clinical, or indeed as yet
40 unrecognised diabetes. Graham et al were silent on diabetes-related matters, but Hoogeveen (2000) reports that tHcy appears to be a stronger (1.9-fold) risk factor for mortality in type 2 diabetic patients than in non-diabetic subjects.

Diabetes incidence in New Zealand is 9.8 per 100,000 for type I (Elliott 1999) while type II diabetes affects adults - up to 20% in some societies. In the western world, cardiac problems
45 cause about 45% of deaths. The World Health Organisation report for 2000 estimates that total deaths caused by diabetes mellitus in 1999 were 777,000 (1.4%), for cardiovascular disease, 17 million (30.3%), and cerebrovascular disease, 5.5 million (9.9%). In the USA, 600,000 people suffer a stroke each year and of these, 160,000 die. The frequency of neural tube defects (NZ: 1991) was 1 in 1750 births, plus unknown miscarriages/terminations.

50 SUPPLEMENTAL VITAMINS and PLASMA HOMOCYST(E)INE (tHcy) LEVELS

Folic acid deficiency at a subclinical level at least is an endemic and under-recognised problem, leading to a number of diseases including VaD. This is partly due to (a) sub-optimal actual dietary levels being common, (b) an original underassessment of actual needs (see for example Rimm (1998), and (c) several common mutations relating to metabolism of folic acid in the
55 general population. The rare inherited disease homocystinuria with hyper-homocystinaemia is associated with childhood onset of cardiovascular occlusive disease. A milder variant of the disease (heat labile methylene tetra hydrofolate reductase) occurs in 10-15% of some populations and also carries a risk of higher than usual VaD. Folic acid deficiency also causes certain defects in early embryonic development such as spina bifida and other neural tube defects. Milk
60 including added folic acid is sold for this purpose as a prophylactic. National programmes for

addition of folic acid to bread and wheat flour now exist, for the "neural tube" reasons in the main. Folic acid can be provided in several forms. Monoglutamates (being more easily taken up by the body) are preferred over natural polyglutamates with a 50% uptake.

Deficiencies of folic acid, pyridoxine, and cobalamin leads to higher tHcy. Graham et al (1997) and many other authors are reminding health authorities of the usefulness of truly adequate dietary supplies of folic acid, pyridoxine, and cobalamin (and in some cases, also with betaine) for reducing tHcy, an important, independent and frequent risk factor for clinical atherosclerosis and venous thrombosis. See for example Brönstrup et al 1998, Ward et al 1997, Graham et al 1997. This supplementation helps even those individuals who are genetically predisposed to hyperhomocyst(e)inemia (Malinow 1999). Graham calculates a reduced risk factor of 0.38 (95% C.I., 0.2-0.72) of cases (of VaD) for individuals within the study found to take supplements containing folic acid, cobalamin, or pyridoxine as compared to non-users = 1, but notes that those individuals may also be more careful about their health in other ways. Furthermore, betaine is capable of reducing tHcy at least in those individuals who have a deficiency of cystathione beta-synthetase activity (Dudman 1996).

There is no absolute value of tHcy that "switches on" vascular disease. The biology of vascular wall disease is still poorly understood but may involve superoxides, and involve sugar-protein condensations in diabetes. Individual tHcy varies with time as well. Renal and thyroid function and certain medications are known to modulate tHcy. (See Pietrzik, 1998).

The commonest group of individuals with folic acid deficiency are the elderly who often have an associated cobalamin deficiency. Correction of the folic acid deficiency without *pari passu* correcting the cobalamin deficiency may produce adverse neurological effects so it is important to correct both deficiencies simultaneously, quite apart from the additional tHcy lowering effects which result from cobalamin treatment. A common genetic variant of cobalamin metabolism has been described which results in an increased dietary requirement for cobalamin and may precipitate relative cobalamin deficiency.

Patent specifications describing a milk-based supplement that includes all three of folic acid, pyridoxine, and cobalamin include US 5985339 Kamarei for a "complete nutritional composition" for use in adult humans against cardiovascular disease amongst a wide range of other diseases (but does not actually refer to tHcy), DE2917239 Saiki teaches a low-calorie complete food, apparently for adults, and US6030650 Kamarei offers a nutritional dairy product, for use in eg ice cream and yoghurt, and independently claims the same with soy milk. For infants, EP951842

Bindels et al is an example general infant formula, topping up amino acids to resemble human milk, and **EP129418 Barr** gives a food for low birth-weight infants. **US 5631271 Serfontein** offers compositions involving pyridoxine biochemistry in particular but with cobalamin and folate. Of the set, only Serfontein refers to hyperhomocysteinaemia, specifically in relation to infants. A composition based on homogenised milk powder for premature infants in the first few days of life, and disadvantages of using cow's milk in relation to methionine metabolism are given.

DIABETES

- 100 Diabetes mellitus is a common endocrine disorder affecting carbohydrate metabolism and blood glucose levels, resulting in substantial morbidity and mortality, and leading to considerable financial costs to individual patients and health care systems. The disease has several forms. Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) is a type of autoimmune disease in which antibodies to islet cells appear and later the production of endogenous insulin is prevented, requiring exogenous insulin treatment for the remainder of the patient's life. Generally the onset is during childhood. Treatment with either insulin or diet and hypoglycaemic drugs provides palliation of the condition but often cannot prevent the feared vascular complications of the disease such as premature coronary heart disease. Type II or maturity-onset diabetes may have a dietary cause.
- 110 That diabetes will cause VaD is well known, although how diabetes acts on the vessel wall is uncertain. There may be common ground with tHcy. Diabetes is also the single largest cause of coronary heart disease. A patient may present with symptoms of heart disease without any obvious manifestations of diabetes and is then diagnosed (for the first time) with diabetes. Vascular occlusive disease of for example the legs, eyes or brain is a common sequel, leading to
- 115 foot gangrene, acquired blindness, and probably stroke. Note that the WHO report does not count diabetes as being an underlying cause of coronary heart disease.

MILK PROTEINS

- Caseins are known to adversely affect some individuals in various ways (including peptide-based effects). The apparent causal relationship of casein types to diabetes incidence is of particular interest. Among some preventive strategies that have been proposed, identification and removal of environmental triggers of the disorders (Popham 1978, Elliott 1999 Padburg 1999 and others), and/or modification of coexistent metabolic conditions have received most attention.

The causative link between casein variants in milk and diabetes itself will now be discussed. Certain fragments (peptides) of beta casein have a structure in which proline residues alternate

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125 with any amino acid, providing resistance to digestion by gut endoproteases may be left intact
and may been found in the circulation after passing through the gut wall. Casomorphins have
that alternating structure. Variants A1 and B of bovine β -casein yield some β -casomorphin 7
(PRO-GLY-PRO-ILE-PRO-GLY) from residues (for type A1) 63 to 68 inclusive, after proteolysis
in the gut. β -casomorphin 7 has opioid-like properties including some action on the gut itself
130 such as on motility, absorbtion, and secretion. It is a direct inhibitor of acetylcholinesterase. Type
A2 β -casein yield β -casomorphin 9, not 7, owing to a further proline residue at site 67 in that
casein. See the structure (sequence) in Fig. 3. Teschemacher (US 4681871) teaches the isolation
and use of various orally active casomorphins, preferably short peptides having opiate like or
analgesic activity, such as β -casomorphin 3 for use in analgesia, but makes no reference to type
135 A2 β -casein nor to functional relationships of casomorphins with diabetes). Elitsur et al discuss
interactions between lymphocytes and casomorphins in the gut wall.

Over the last 15 years or so, the numerical association both between and within countries and
their societies (Padberg 1999) relating ingestion of cows' milk or products thereof and onset of
diseases such as Type 1 diabetes has been studied. Critical analysis by the inventor - see Elliott
140 (1999) - has identified a strong correlation between the incidence of Type 1 diabetes and a
weighted average of consumption of β -casein types A1 and B (that is, excluding type A2). This
comparison copes with the anomalous Icelandic statistics (high milk consumption; low incidence).
Icelandic cows secrete A2 almost alone.

WO96/14577 teaches that milk protein genes are expressed in a co-dominant way, so that
145 individual phenotypes typically result in mixed caseins including β -casein mixtures such as A1A2,
A2A3, A2B, and so on: there being a number of alleles in existence. Gene frequencies vary
between breeds, with Holstein/Friesian tending to be low in type A2 variant alleles. That appli-
cation teaches that for use in dairy production, selection of only those cows that have an A2A2
genotype and produce only the β -casein A2 variant, (not A1 nor A1A2 nor B) or alternatively the
150 consumption of dairy products not containing β -casein A1 will tend to reduce the incidence of
diabetes. The presumption has been made in the prior art that the diabetogenic effect of milk
including β -casein A1 (or the like) is solely a result of the β -casomorphin 7 inevitably released
during proteolysis in the gut. Monetini et al describe finding significantly increased levels of
antibodies to β -casein in patients with type I diabetes.

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190 OBJECT

It is an object of this invention to provide a fortified dietary supplement capable of reducing vascular disease and/or diabetes, or at least to give the public a useful choice.

STATEMENT OF INVENTION

195 In a first broad aspect the invention provides a fortified dietary supplement comprising a milk or milk product wherein the dietary supplement is fortified by addition of an effective amount of at least one compound selected from the group (known herein as Group I) that includes betaine, cobalamin, folic acid, pyridoxine, and pharmaceutically acceptable analogues of each substance; the fortified dietary supplement, when consumed, being capable of reducing plasma levels of homocyst(e)ine (tHcy) so being capable of reducing the incidence of vascular disease (VaD),
200 particularly cardiovascular disease and cerebrovascular disease, and NTD in a mammalian population.

In a closely related aspect, the invention provides a dietary supplement wherein the supplement is fortified by addition of an effective amount of each of at least two compounds selected from Group I.

205 More preferably the invention provides a fortified dietary supplement comprising a milk or milk product wherein the selection from group I includes folic acid (or a pharmaceutically acceptable analogue thereof) and at least one other member.

Preferred concentrations of mixtures of any two or more of the above compounds is assumed to be the same amount for each as if each was used separately.

210 Preferably, folic acid is used at least along with cobalamin.

In a related aspect, the invention provides for the use, in the manufacture of a dietary supplement, of an effective amount of at least one compound selected from Group I together with milk or milk products; the dietary supplement being intended for effecting a reduction of tHcy and indirectly the reduction of VaD in a population.

215 A preferred range of amounts of folic acid supplementation suitable for an adult human is from about 300 to about 500 micrograms (μg) intake per day; more preferably 400 micrograms, and assuming a daily intake of 400 ml of milk, this corresponds to 1 microgram of folic acid or the pharmaceutically equivalent amount thereof of an analogue, per ml of milk. Preferably this preparation provides an acceptable way of controlling the incidence of neural tube defects in a

220 population.

A preferred range of amounts of cobalamin is from about 4 to about 7 μg per day; more preferably 5 μg , and assuming a daily intake of 400 ml of milk, this corresponds to 0.012 μg cobalamin, or the equivalent thereof per ml of milk. Optionally an increased amount may be provided in cases of malabsorption.

225 A preferred range of amounts of pyridoxine is from about 1.5 to about 4 mg per day; more preferably 2 mg, and assuming a daily intake of 400 ml of milk, this corresponds to 5 μg pyridoxine or the equivalent thereof per ml of milk.

At least one further compound capable of reducing tHcy is betaine, and a preferred effective amount as a daily intake of betaine is up to 1 g per day; more preferably about 100 mg per day,
230 preferably together with the other specified compounds.

In a second broad aspect, the invention provides a dietary supplement comprising a milk or milk product fortified as previously described in this section, wherein the milk or milk product further has a controlled bovine beta casein content substantially comprised of the A2 variant, so that the dietary supplement is capable of reducing the incidence and/or effects of vascular disease (VaD)
235 in the population, both as a result of reducing tHcy by means of the added substances as previously described in this section, and as a result of reducing the incidence of Type 1 and Type 2 diabetes through modifications of the casein composition.

In a related aspect the invention provides for the use, in the manufacture of a dietary supplement, of milk or milk products of bovine origin which are characterised by a substantial absence of (at
240 least) type A1 or type B β -casein; the dietary supplement being intended for the reduction of the effects of diabetes in a population and, as a result, reduction of the effects of VaD in the population.

In another related aspect the invention provides for the use, in the manufacture of a fortified dietary supplement, of milk or milk products of bovine origin which are characterised by a
245 substantial exclusion of type A1 or type B β -casein and by the addition of an effective amount of at least one compound selected from Group I: the dietary supplement being intended for the reduction of the effects of diabetes in a population and, as a result, tending to reduce the effects of VaD in the population.

Preferably the dietary supplement includes type A2 β -casein.

250 In a third broad aspect, the invention provides a dietary supplement comprising a milk or milk product fortified as previously described in this section, wherein the milk or milk product further has a bovine origin, has a controlled beta casein content substantially comprised of the A2 variant, and has an immunological property at least made evident during a process of digestion, whereupon at least some of the β -casein A2 is converted into a relatively stable active compound
255 capable of promoting immunity against diabetes by means of an action occurring in or about the wall of the gut.

In a related aspect this invention provides a relatively stable active compound capable of promoting immunity against diabetes; the compound comprising a peptide which is relatively stable in the gut in the presence of digestive enzymes, and preferably the peptide includes from
260 seven to twelve amino acid residues, wherein proline makes up a large proportion of the residues.

In a further related aspect the invention provides a dietary supplement as previously described in this section, wherein the relatively stable active compound is a peptide having more than seven amino acid residues, and more particularly the relatively stable active compound is the peptide known as β -casomorphin 9, having a peptide sequence as shown in Fig 3 and the compound has
265 an ability to cause an at least partial protection from diabetes.

Alternative caseins include caseins derived from other species of mammal.

Alternatively an active compound based on the structure of bovine β -casomorphin 9 may be made by recombinant means, or from casein by proteolysis, or be synthesised as a peptide or as pharmaceutically acceptable salts thereof or as pharmaceutically acceptable esters thereof, and
270 supplied as part of a dietary supplement in a stabilised form.

Preferably the relatively stable active compound capable of promoting immunity against diabetes is assisted by the inclusion of at least one agent having an adjuvant-like effect within the dietary supplement, the agent being capable of enhancing a development of immunity. Optionally the stabilised form allows a slow release of the active compound so that it is released into the gut
275 over a period of time.

In a fourth broad aspect this invention provides a fortified dietary supplement capable of removing two risk factors associated with diabetes and VaD; the fortified dietary supplement comprising a combination of a cow milk or cow milk product substantially free of A1 and B casein, together with an effective amount of at least one compound selected from Group I.

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280 In a related aspect the invention provides a fortified dietary supplement including at least one compound as previously described in this section, and A2 casein; the product being capable of improving the status of the cardiovascular system and of lowering the risk of initiating a diabetic condition.

285 In a fifth broad aspect this invention provides a method for preparing a fortified milk product as claimed in any previous claim, including the steps of providing a milk having a specified composition of casein as previously described in this section, optionally pasteurising or otherwise sterilising the milk, and of adding sufficient of at least one compound selected from Group I to reach an effective final concentration of each compound as previously described in this section.

Optionally the milk is treated so as to become substantially free of type A1 or type B beta-casein.

290 In a sixth broad aspect this invention provides a method for minimising the incidence and/or the effects of the disease diabetes mellitus comprising the steps of using, in a diet, an effective amount of a fortified milk product as previously described in this section.

In a seventh broad aspect this invention provides a method for minimising the incidence and/or the effects of VaD in a population comprising the steps of using, in a diet, an effective amount of
295 a fortified milk product as previously described in this section. In a related aspect this invention also provides a method for minimising the incidence and/or the effects of NTD in a population.

Preferably the fortified milk product replaces any unfortified milk product in the diet.

Optionally the fortified milk product has an altered casein composition as previously described in this section

300 In an eighth broad aspect this invention provides a fortified milk product including an effective amount of at least one compound selected from Group I.

In a first related aspect this invention provides a fortified milk product made from milk of the A2 β -casein type so that substantially no type A1 β -casein is present, and preferably also so that substantially no type B β -casein is present. Alternatively the caseins may be specified as
305 "substantially no casein capable of yielding a beta-casomorphin-7 upon digestion in the gut is included in the fortified milk product".

In a ninth broad aspect this invention provides a method for reducing the incidence in a population of at least one of: (a) diabetes type I, (b) diabetes type II, (c) cardiovascular disease, (d) cerebrovascular disease, (e) peripheral vascular disease, or (f) degeneration of blood vessel walls,

310 comprising the steps of manufacturing and providing to the population a dietary supplement in the form of a milk product including A2 beta-casein but substantially no A1 or B beta-casein, and fortified by addition of an effective amount of at least one compound selected from Group I. Preferably further diseases as listed in this specification under "Other diseases" are also covered.

315 In a tenth broad aspect this invention provides a method for the minimisation of Type I diabetes by providing that a population at risk shall be provided with identified dairy products obtained from breeds or strains of dairy animal that produce β -casein A2 only and substantially no β -casein A1 nor β -casein B so that members of the population have the opportunity to consume the identified dairy products and so that the individuals become protected by exposure to a therapeutically effective amount of β -casomorphin 9.

320 In a related aspect this invention provides a method for the minimisation of Type I diabetes by the oral administration of dietary preparations including added β -casomorphin 9 or precursors thereof.

In an eleventh broad aspect this invention provides for the use in a dairy industry, or at least in some commercial aspect thereof, of breeds or strains of dairy animal that produce β -casein A2
325 and substantially no β -casein A1 nor β -casein B.

In a twelfth broad aspect this invention provides a method for the creation, by a process of selection from a mixed population of breeds or strains of dairy animal that produce β -casein A2 and substantially no β -casein A1 or A1A2 nor β -casein B wherein the method comprises appropriate animal selection or animal separation methods known in the art, so that dairy products
330 confirmed as having substantially only type A2 casein are produced.

In a thirteenth broad aspect this invention provides a dairy product having undergone purification during a manufacturing process, so as to eliminate at least β -casein A1, if not also β -casein B, and even all casein, from a product.

In a fourteenth broad aspect this invention provides a milk, a milk-based product, or a dairy
335 product having, as a result of selection of contributing cows, a reduced concentration of at least β -casein A1, if not also β -casein B. In a related aspect this invention provides a milk, a milk-based product, or a dairy product having, as a result of a subsequent manufacturing process, a reduced concentration of at least β -casein A1, if not also β -casein B.

In a fifteenth broad aspect this invention provides a milk-free surrogate product fortified with an effective daily replacement of at least one compound selected from Group I as previously described in this section, so that those unable for any reasons to consume dairy products can nevertheless receive regular tHcy-reducing supplementation with their artificial eg soy milks.

In a sixteenth broad aspect the invention provides a dietary supplement for the treatment of mammals other than humans (such as cats or dogs, which can develop diabetes).

345 **PREFERRED EMBODIMENT**

The description(s) of the invention to be provided herein is/are given purely by way of example and are not to be taken in any way as limiting the scope or extent of the invention.

DRAWINGS

Fig 1: Graph of results of the BB rat/ Prosobee trial.

350 Fig 2: International correlations between A1 beta casein and rates for four diseases.

Fig 3: Sequence of bovine beta casein A2 with the position of the casomorphin indicated.

PRINCIPLES

Milk is commonly and extensively consumed in many societies where the risk and incidence of both diabetes and VaD is high. Existing forms of milk may contribute to both diseases; there is published and unpublished epidemiological evidence. A diet with milk including type A1 beta casein or type B beta casein is closely correlated with Type I diabetes; while milk containing only type A2 beta casein is not (Elliott, 1999). Perhaps this dietary threat can be converted into an opportunity, replacing or supplementing health-adverse components of milk with health-promoting components. Then, the population at risk simply may substitute one type of milk for another.

360 Aspects of this specification include (1) proposing a theory that β -casomorphin 7 apparently tends to cause diabetes, (2) proposing a theory that β -casomorphin 9 may be used in an immunisation procedure, and (3) proposing that the supply, to a population, of milk including only the β -casein A2 variant optionally together with tHcy-reducing compounds will significantly reduce diabetes and vascular disease.

This specification concentrates on the disease processes having the vascular wall as a common point of action (herein called Vascular Disease or VaD). A combined treatment including both the diabetes and the (tHcy) strategies for control of vascular wall diseases will exhibit an additive and possibly even a synergistic extent of action. That Graham (et al) 1997 observe multiplicative effects between (tHcy) and for example smoking is noted. No trials of the invention have yet been carried out and it is more likely that results which are significant on a population basis can only result from actual use of the invention. Woo et al (1999), describe measured improvement in relation to repeated scans of the brachial artery during and after folic acid treatment, and Hackam et al (2000) describe measured improvement in relation to carotid sinus/carotid artery plaques in adults having reduced tHcy after supplementation of folic acid over some weeks.

Prophylactic milks and milk compositions according to the invention are intended to reduce VaD incidence, directly through the use of tHcy reducing agents, and indirectly by reducing the incidence of diabetes through (a) provision of bovine milks high in the A2 variant of beta-casein and low in A1 and B variants, and/or (b) exploitation of the immunological properties of beta-casomorphin 9 (an active and relatively stable peptide digest fraction of A2 beta-casein, having nine amino acids). Reduction of tHcy levels, highly correlated with improved vascular wall health, is accomplished through fortified dietary formulations, comprising milks having casein variants as above, having an effective amount of at least one compound selected from the group of betaine, cobalamin, folic acid, and vitamin B6 (pyridoxine). Practical fortified diets which will be widely consumed by a population at risk include treated, selected milks, and also selected milks together with treated cereals. Fortified milk products such as ice cream, yoghurt, dried milk powder, and the like may be added.

EXPERIMENT 1

This study involved "BioBreeding" (BB) rats in a trial. See Fig 1 for a graphical display of the results with diabetes incidence (1.0 = 100%) on the vertical axis. The control diet was "Prosobee" (TM) which is a soy preparation used as rat food in laboratories. The spontaneous incidence of diabetes in the control population was about 38%. Rats fed "Prosobee" (TM) plus 10% mixed casein (that is, A1 and A2) had an incidence of about 27%. Rats fed on "Prosobee" (TM) plus 10% type A1 casein had an incidence of about 45%. Rats fed on "Prosobee" (TM) plus 10% type A2 casein had an incidence of about 20%. In accordance with the teaching of WO96/14577) the incidence of diabetes in the A1 group was higher than that of the control group.

Had the A2 casein (or breakdown products thereof) been simply a "neutral" substance, then the incidence of diabetes in the A2 group would be about the same as that of the control group, because the adverse effects of β -casomorphin 7 are absent. In fact the incidence of diabetes in the A2 group was significantly reduced and was the lowest of any group. Therefore the inventor proposes that β -casomorphin 9 exerts a beneficial effect on the incidence of Type I diabetes. Presumably it acts as an immunomodulator. The actual mechanism of action whereby a casein fragment from the milk of one species of animal has an effect on antibodies against β -islet cells of the pancreas in at least humans and susceptible laboratory rodents is unknown but it has been observed that caseins are involved in cellular structures and small peptides such as casomorphins may act as intracellular messengers.

EXPERIMENT 2

The effects of two peptides derived from beta casein were studied by comparing the results in normal (SWR/J) mice and NOD(diabetes prone) mice. The two peptides were: β -casomorphin-7 which is an enzyme digestion product of A1 beta casein, but not of the A2 variant, or β -casomorphin-9, which is a digestion product of A2 beta casein, but not of the A1 variant. Female mice, age 30 days had been fed on a milk protein free diet since weaning. There were ten animals in each group. At day 30 they were injected with 10 mg ovalbumin in an adjuvant (Freund's complete) and 1 mg of one or other of the peptides. The peptide injections were repeated daily for a further ten days. Blood was taken from the animals on day 7 and 11 and analysed for IgG and IgM antibodies to ovalbumin.

Results: The NOD mice were characterised by a lower IgM and IgG response at 7 days compared with the control animals. This persisted to 11 days in the case of IgM but not the IgG. The mean immunoglobulin responses to the ovalbumin were greater in the normal mice given either β -casomorphin-7 or 9 than saline treated controls at 7 but not 11 days.(Table 1)

Table 1; Normal mice: mean delta OD from saline controls

7 days	β -casomorphin-7	β -casomorphin-9
IgG	<u>0.118</u>	<u>0.106</u>
IgM	0.130	0.201
11 days		
IgG	0.050	0.050
IgM	0.020	0.030

Both peptides accelerate the early immune responses with β -casomorphin-9 possibly having a

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greater effect than β -casomorphin-7.

On the other hand this early peptide enhancement of antibody responses were not seen in the
425 NOD mice at 7 days, and only for BCM IgG responses at 11 days (Table2).

Table 2; NOD mice: mean delta OD from saline controls

7 days	β -casomorphin-7	β -casomorphin-9
IgG	<u>0.006</u>	<u>0.038</u>
IgM	0.007	-0.002
11 days		
IgG	0.102	-0.092
IgM	-0.018	-0.007

These experiments establish that in the diabetes model (the NOD mouse) there is a deficient
early antibody response to parenterally administered antigen which is not corrected by either of
the co-administered milk peptides. However the early stimulatory effect on IgG levels in the
430 SWR/J animals of β -casomorphin-9 is relatively better retained than in the NOD mice.

Milks yielding β -casomorphin-9 may ameliorate the early antibody deficient response
(NOD/SWR/J ratio =0.36) than milks which yield β -casomorphin-7 (NOD/SWR/J ratio = 0.05).
The slight "advantage" from β -casomorphin-9 may reflect the mechanism of diabetes causation.
Of course these experiments are based on injected, not gut-accessed antigens.

435 **EXPERIMENT 3**

Fig 2 summarises the results of this experiment: a retrospective survey of information to look for
a relationship between the incidence of some selected diseases and the amount of milk drunk per
capita in a population and more specifically the amount of A1 beta casein consumed per capita.
This figure was calculated using the A1 beta casein proportion for each breed of dairy cow
440 contributing to the milk in a given country, and the proportion of the national herd comprised of
each breed, using the dairy science literature for each country. Milk protein consumption was
obtained from the FAO web site. Data for the incidence of diabetes was restricted to white
populations in order to reduce confounding owing to ethnic-related genetic variations in Type I
diabetes. Data for asthma prevalence was based on a "12 months prevalence of wheeze" as
445 published by the International Study of Asthma and Allergies in Childhood. Data for the
incidence of multiple myeloma was obtained from cancer registry data circa 1990.

This experiment is an ecological study, with all accompanying limitations. We observe that A1

casein in the per-capita food supply across countries (circa 1980) was significantly correlated with (1) the incidence of diabetes in childhood over the same time, (2) ischaemic heart disease mortality in adults in 1990, (3) incidence of multiple myeloma in males and females in 1990, and that the rates of these diseases were correlated with each other. (Possibly the biological basis of myeloma is related to lymphocyte suppression by beta-casomorphin 7 from A1 milk.) Even though some statistically significant correlations have been found, this is not proof of causation, although that possibility is raised. Nevertheless, the changed value and significance of the correlation between (all milk) and (A1 beta casein) for type I (IDDM) diabetes and for ischaemic heart disease (CHD) must be explained. We also note that if B beta casein was added with A1 to the consumption, the correlations became weaker. This study plus the two animal experiments, supports the claims of this invention to the health-conferring properties of milks or milk products having low or no type A1 beta casein, even without the extra health-conferring properties of the Group I supplements.

BEST METHOD FOR CARRYING OUT THE INVENTION

The invention proposes various combinations of milk comprised of predominantly type A2 casein, or the like. EXAMPLE A is a fortified dietary supplement including effective amounts of added folic acid, together with B6 and cobalamin which are known to have effects on tHcy; the supplement being based on ordinary milk. EXAMPLE B has "controlled casein compositions". The combination (EXAMPLE C) of (A) and (B) is expected to show additive effects if not actual synergy at the level of pathophysiological effects on blood vessel walls and the like in patients with known or unsuspected diabetes, or homocyst(e)inaemia. Cures may occur.

Example A alone is generally useful in minimising the effects of diseases other than diabetes, herein being the group known as "VaD". In such cases the controlled casein example (B) of the invention has at least no undesirable effects and may actually reduce the onset of diabetes in at-risk persons. Note that the incidence of "unsuspected diabetes" in patients having a vascular disease is surprisingly high.

ILLUSTRATIVE EXAMPLE (A)

This example describes the fortifying of any commercially produced milk with an effective amount of at least one compound selected from the group of betaine, cobalamin, folic acid, and vitamin B6, for reducing tHcy. The preferred prescribed amounts of the fortifying materials are determined in relation to known "recommended daily amounts" (RDAs) together with reports

from the literature about tHcy, and the likely daily consumption of the fortified milks or milk products by a typical consumer. (The LD₅₀ factors for the added materials is high). Relevant "quantity-setting" references include: (A) Lobo et al (1999) in Ohio found 1 mg folic acid per day (in combination with B6 and cobalamin) no better than 400 micrograms re tHcy, (95 patients of a mean of 61 years of age), (B) Ward et al (1997) for reducing tHcy in young men in Northern Ireland found 400 micrograms no better than 200 micrograms but 200 was more effective than 100 micrograms (30 volunteers over 26 weeks), (C) Brönstrup et al (1998) in young German women found that 400 micrograms folic acid together with cobalamin (either 6 or 400 micrograms) was significantly more effective on tHcy levels than folic acid alone. Further, the recommended daily dose of folate for avoidance of neural tube defects is about 400 micrograms a day. One can conclude that an effective supplementary dose of folic acid is around 400 micrograms a day, but these trials use ordinary people and would not have involved an otherwise totally folate-free environment. Hence we nominate from 300 to 500 micrograms (mg) intake per day of folic acid suitable as a daily dose for an adult human (if comprising the single added compound). Assuming a daily intake of 400 ml of milk, this corresponds to about 1 microgram folic acid or the equivalent thereof per ml of milk.

Less clear evidence is available for setting preferred range of amounts of cobalamin (whether or not it is a single added compound). At this time we prefer from 4 to 7 micrograms per day. 5 micrograms corresponds to 0.012 µg cobalamin per ml. (assuming no absorption defects in the recipient). Similarly for pyridoxine the preferred range is from 1.5 to 4 mg per day. 2 mg daily within 400 ml of milk corresponds to 5 µg pyridoxine or the equivalent thereof per ml of milk.

Betaine is also known to be capable of reducing tHcy. A preferred effective daily intake is up to 1 g per day; more preferably about 100 mg per day, preferably together with the other specified compounds. Analogues of all these constituents are well known in the pharmacological arts and corresponding effective doses may be prescribed. Mixtures of two or more of the above compounds are preferred because of evidence of increased efficacy (for example see Brönstrup et al (1998)) and for each, the amount is assumed to be the same as if each was used separately. Note that cobalamin in amounts similar to the average daily requirement (5 micrograms) should be taken if folic acid is administered. This amount will prevent adverse effects of folic acid (such as on brain function) when given in the above doses to individuals (such as the elderly, with absorption deficits) who may be deficient in cobalamin.

Preferably such vitamin fortifications are added by mensurated line feeder methods familiar to those skilled in the art, prior to making the milk available for consumption or further

manufacture into milk products. Although cobalamin and B6 are degraded by light, they are not notably heat-sensitive and will survive the usual pasteurisation. Indeed, they may survive extensive processing such as drying. Preferably such milk /milk products are analysed for verification of the vitamin additions prior to being consumed, by methods familiar to those skilled in the art. The liquid milk thus fortified may be of any of the commercial presentations of milk including but not limited to fat reduced milk, ultra heat treated milk or pasteurised milk. The invention also applies to "milk products" as previously defined.

One obstacle to the widespread adoption of this "automatic administration" route to reduced tHcy in a population is that some individuals cannot drink milk (or eat products made from milk) for medical reasons such as an allergic response, and some societies do not drink milk for cultural, non-availability, or religious reasons. Therefore this invention proposes to make available to such people or societies several options of fortified liquids that are usually drunk frequently instead of milk. Accordingly, the invention also consists in (1) a fortified soy milk or the like, (2) a "tea/coffee additive" probably water or perhaps a fortified sweetener, (3) a fortified carbonated beverage, and (4) bottled fortified drinking water, each including at least one compound selected from Group I; preferably at least folic acid and cobalamin, having concentrations to give most users an adequate daily dose.

ILLUSTRATIVE EXAMPLE (B)

This aspect of the invention adds to the teaching of Example A in relation to the prevention of VaD with further information relating to the prevention of diabetes, both Type 1 and Type 2, by provision of cow milk or milk products which are substantially free of those proteins (caseins) which are capable of yielding β -casomorphin-7 or other longer peptides containing the beta-casomorphin-7 sequence, after intestinal digestion in the recipient mammal (including man). This may be accomplished by

- a) selection for a dedicated milk supply of cows which yield beta casein only of the A2 variety to provide the milk - such milk not yielding the β -casomorphin-7 or related peptides after intestinal digestion. There are in existence cows which produce A1, A2, or B casein in a mixed fashion reflecting proteins expressed as a result of the existence of several codominant genes, and selection means well known in the dairy arts can be used to (1) breed selectively for animals providing A2 caseins alone (bull selection is one "short cut" to achieve rapid change of the genetic makeup of a population of cattle given the availability of artificial insemination) (2) pick out of a mixed population those animals that are homozygous for A2, and provide

quality assurance procedures on products:

- 545 b) (optionally) removal of all or substantially all beta-caseins from milk by physical, chemical or enzymatic means, or just the A1 and B beta-caseins leaving the A2 casein,
- c) (optionally) genetically altering the milk source cows such that the beta-caseins which yield β -casomorphin-7 or related peptides do not occur after digestion. Such genetic alteration may totally remove the required gene sequence for either the specific, or all, beta casein.
- 550 Selection of the cows producing the required milk involves identification by measurement of the various beta caseins in individual milk samples and using only those cows producing A2 beta-casein. The beta casein variants may be identified by gel electrophoresis or other methods familiar to those skilled in the art.

We believe that the invention may be explained in theory by discoveries as follows.

555 **Part 1: The nature of a dietary environmental agent which can trigger diabetes.**

Previous research has shown an epidemiological association between the quantity of consumption of liquid milk and Type 1 diabetes (Scott et al), and that the probable cause of this association can be further apportioned to the A1 and B beta casein moieties of the milk (Elliott et al 1999, Laugesen et al (unpublished)). Other milk proteins are not known to be associated with diabetes.

- 560 In particular the A2 variant of beta casein appears to be harmless. Other research has shown an association between Type 1 diabetes and high levels of antibodies to A1, but not A2 beta-casein - at least in some populations (Elliott et al 1999).

- 565 Virtanen et al (2000) have shown that children who develop diabetes drink more cow milk than those who do not, when the genetic predisposition to diabetes is taken into account. Infants born with a genetic predisposition to diabetes and fed a diet containing no cow milk in infancy are less likely to develop early signs of disease affecting the insulin producing cells of the body than those fed cow milk. (Akerblom et al 1999). Bennett et al have shown an association with the early childhood consumption of cow milk and Type 2 diabetes. Thus in humans it appears that milk consumption may be an environmental trigger to both types of diabetes and at least in Type 1
- 570 diabetes this appears to be associated with the content of A1 and B beta caseins of the milk - but not the A2 beta casein content.

Both A1 and B beta caseins yield beta-casomorphin-7 after digestion by intestinal digestive enzymes, whereas A2 beta casein does not. β -casomorphin-7 has opiate type effects on intestinal

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transit time in animals (including humans) and also has immunosuppressive activity on human
575 intestinal lymphocytes (Elitsur 1992). Such opiate like effects may exacerbate a genetic
predisposition to Type 1 and Type 2 diabetes.

Part 2: The (known) association of coronary heart disease with diabetes. Both Type 1 and Type 2
diabetes increase the risk of coronary heart disease 5-10 fold. See Fig 2. In some communities
Type 2 diabetes occurs in greater than 10% of the adult population over the age of 40, and in
580 these communities diabetes is the leading cause of coronary heart disease. Type 1 diabetes has a
smaller contribution to the population coronary heart disease rate. Diabetes incidence (both
types) is increasing dramatically throughout the world.

**Part 3: The association of coronary heart disease with the consumption of liquid milk and in
particular milks containing the A1 and B variants of beta casein.** Several epidemiological studies
585 cited above (see Fig 2) have shown an association between the consumption of liquid milk and
coronary heart disease mortality rates and this appears to be due to the protein content of the
milk rather than the fat content. As can be anticipated, given the association of diabetes with
heart disease, the consumption of A1 beta casein appears to be better associated with coronary
heart disease mortality rates than is any other constituent of the cow milk.

590 **Part 4: Is there an actual link between tHcy and diabetes?** Hoogeveen (2000) reported that tHcy
is related to 5-year mortality independent of other major risk factors and appears to be a stronger
(1.9-fold) risk factor for mortality in type 2 diabetic patients than in non-diabetic subjects.

Part 5: The association of elevated tHcy with increased cardiovascular mortality. The literature
which has been reviewed in this specification leads one to conclude that the most effective way in
595 which high tHcy can be lowered is by giving all three vitamins - and betaine. The resulting
lowered VaD risk may possibly operate through the mechanism of reducing vascular resistance
and thrombotic tendency. An additional useful (though unrelated) result of such additions (of the
folate in particular) is reduction of the occurrence of the neural tube closure defect spina bifida
and related defects if these vitamin supplements are consumed during early pregnancy.

600 **Part 6. Possible immunological mechanisms** whereby one particular peptide from casein, β -
casomorphin 9 appears to be beneficial though an immunological property, while β -casomorphin
7, from β -caseins type A1 and B appears to be the harmful component of some milks. One
characteristic of the casomorphins is that the alternating proline residues will "protect" adjacent
peptide bonds from attack by endopeptidases. Caseins happen to include repeated alternating

605 proline - X residue sequences. There is only the one sustained sequence - that at residue 60 to
68, although another pro-tyr-pro-glu sequence is at residue 180. The alternative explanation is
based on the discovery that the peptide β -casomorphin 9 matches amino acid residues number 60
to number 68 inclusive of bovine β -casein A2 (See Fig 3). The composition of this peptide
confers resistance against further digestion using cleavage by endopeptidases. This peptide is
610 released into the gut during the digestion of milk containing that variant of casein.

The peptide β -casomorphin-9 is believed to have an immunoprotective effect or at least an
immunomodulatory effect in relation to Type I diabetes and as a result the consumption of milk
including β -casein A2 (and substantially no β -casein A1 nor β -casein B) will result in a reduction
in the incidence of diabetes to below the rate of incidence in a control population. Some experi-
615 ments intended to show immunological activity of β -casomorphin 9 will now be described.

ILLUSTRATIVE EXAMPLE (A) + (B)

Further examples of the fortified dietary supplement include:

1. A liquid milk having a type A2 casein composition; substantially lacking either type A1 or type
B casein together with added compounds from Group I (see definitions). Preferably at least
620 two compounds are added to take advantage of inter-relationships and a preferred amount in a
daily intake is the accepted human daily requirement, namely about 400 micrograms (μ g) folic
acid, 5 μ g of cobalamin and 2 mg of B6. Assuming that the average daily intake of milk is 400
ml, a concentration of 100 μ g folic acid, 1.25 μ g cobalamin, and 0.5 mg B6 per 100 ml of milk
having type A2 casein but no type A1 casein supplies this intake. Note that we are aware of
625 special cases such as the elderly who may require extra cobalamin to compensate for poor
absorbtion. For these special cases, a special fortified dairy product with extra cobalamin, or
with an intrinsic factor, may be provided. Adjustments may be made to other components.
2. Provide a wheat flour supplemented with folic acid or analogues thereof. Other members of
the tHcy reducing group may be added; however it may be wasteful to provide cobalamin in
630 that way. Some individuals cannot eat wheat flour or at least tolerate the gluteins.
3. An alternative (in terms of securing the goal of a reliable daily intake) is a fortified breakfast
cereal, having additional compounds according to the invention, to supply a daily intake as
above, sold together with a container of suitably preserved A2-casein milk as a "kit of parts".

635 This may comprise an amount of "UHT" or otherwise long-life milk in a sachet, and the combination might be sold or dispensed as single "ready-to-use" breakfast amounts of cereal and corresponding milk.

4. Fortified ice creams, yoghurts, etc made from milks of the A2 casein type.
5. Other food products, not obviously dairy in type, but made with casein.
6. Infant milk, suitable for even very young infants, possibly fortified to a lesser extent and made
640 from milks of the A2 casein type.
7. Milk products designed for acceptability by young people, fortified with vitamins and made from milks of the A2 casein type. (Young women who are as yet unaware of their pregnancy are at risk of spina bifida damage - which occurs at an early stage of embryogenesis). Arterial lesions are not unknown in young people.
- 645 8. Any one of the entire range of edible milk-based products such as milk powder, milk chocolate, cheese, and the like, fortified with compounds according to the invention and made from milks of the A2 casein type.

650 Water, then wheat flour, then milk seem to be the three most predictable components of the average Western diet. Cereals are very often the "standard breakfast. Hence we select those components so that an adequate daily intake is achieved without special effort - no need to remember to take pills or the like. Most probably the various products suggested in this specification would be offered for sale as "heart-friendly" or the like alternatives.

655 Heat stability of natural folic acid is poor, but artificial folates or combinations will tolerate pasteurisation for example with minimal loss. Light stability of cobalamin (and B6) in milk is poor, hence any product according to the invention should preferably be stored away from sunlight.

660 The rationale for this preventive treatment approach is a combination of removal of a dietary factor found in cow milk and implicated in both diabetes and coronary heart disease, and fortification of milk or milk products rendered free of the adverse dietary factor, with a combination of B group vitamins and/or betaine, which are capable of lowering tHcy. Methods of producing the individual properties of such a milk and products are known to those familiar with the art, but the combination of such properties with the intention of preventing occlusive vascular disease is unique to this invention.

In order to make use of this discovery, many avenues may be explored. For instance...

- 665 1. Use, in a dairy industry of breeds or strains of dairy animal that produce β -casein A2 and substantially no β -casein A1 nor β -casein B. (Examples: the *Bos indicus* subspecies, and the dairy cows existing in Iceland, goats, or even humans, where use of the latter may avoid exposure to certain types of cows milk or products thereof particularly in early postnatal life).
- 670 2. Selection, from a mixed population, of breeds or strains of dairy animal that produce β -casein A2 and substantially no β -casein A1, A1A2, or B using some or all of the selection methods known in the art. For example, cows may be herd-tested for casein variants secreted and those producing other than the A2 variant rejected. Bulls under consideration as AI sires will be either directly tested using methods of genetic engineering, or daughters (preferably bred from A2 type dams) from the initial proving progeny will be tested as above.
- 675 3. Consumption, at least by individuals known to be susceptible to Type I diabetes, of dairy products only from those breeds or strains of dairy animal that produce β -casein A2 and substantially no β -casein A1 or B.
- 680 4. Administration of preparations including β -casomorphin 9 orally, along with foodstuffs whether of dairy type or not. The β -casomorphin 9 may be made by recombinant means, from casein, or may be synthesised.
5. Administration, perhaps in a slow-release formulation so as to promote or extend an immune response of β -casomorphin 9.
6. Administration of "helper materials" which may include...
 - 685 (a) substances to enhance the enzymic cleavage of β -casomorphin 9 from proteins,
 - (b) substances to help carry β -casomorphin 9 into at least the lamina propria of the gut,
 - (c) substances that enhance the response of immunologically capable cells within the body to the presence of β -casomorphin 9.
- 690 7. Any of the above strategies but instead employing an improved peptide or the like having enhanced capabilities in terms of conferring resistance to type I diabetes over those possessed by β -casomorphin 9. (While β -casomorphin 9 is a naturally occurring peptide having desired activity, further research may lead to more active materials possibly with less adverse effects.

COMMERCIAL BENEFITS or ADVANTAGES

Given the incidence of heart and other vascular disease and the difficulty of reliably diagnosing diabetes, plus the marginal status of many people in relation to folic acid intake requirements, it is evident that this invention could, with little inconvenience and without great difficulty of cost or manufacture, save many of the institutional (treatment) costs and personal costs associated with the wide-spread diseases, diabetes, and VaD.

For convenience the invention could be marketed as an alternative type of milk, perhaps called "Heart milk", used just like ordinary milk and preferably as a complete substitute so that the daily dose is assured.. The additives are sufficiently heat stable to survive use in tea or coffee. Further, some specific benefits of this invention include:

- (1) A reduction in the population incidence of at least Type I diabetes. This would avoid a good deal of human suffering and in New Zealand (pop. 3.8 million) would save about one million dollars over the lifetime of each affected person.
 - (2) There is less information available about type II diabetes but benefit to patients would accrue from the tHcy reduction.
 - (3) VaD reduction as expressed in heart disease mortality, stroke morbidity and mortality, amputations as a result of peripheral vascular disease, kidney transplants, and so on.
 - (4) The product provides sufficient daily folate to avoid neural tube defects and if acceptable as a kind of milk to all women of child-bearing age, use of the product should eliminate that problem from a population.
 - (5) Acceptance of the role of the variants of casein will allow an improved national herd to be built up by selection based on a rational premise.
 - (6) The health of a human population can be improved without actual medication.
- Finally, it will be understood that the scope of this invention as described and/or illustrated within this specification is not limited to the preferred embodiments described herein for illustrative purposes. Those skilled in the art will appreciate that various modifications, additions, and substitutions are possible without departing from the scope and spirit of the invention as set forth in the following claims.

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720 **CLAIMS**

1. A dietary supplement comprising a milk or milk product: *characterised in that* the dietary supplement is fortified by addition of an effective amount of at least one compound selected from the group (known herein as Group I) that includes betaine, cobalamin, folic acid, pyridoxine, and pharmaceutically acceptable analogues of each substance; the fortified dietary supplement, when consumed, being capable of reducing plasma levels of homocyst(e)ine (tHcy) so being capable of reducing the incidence of vascular disease (VaD), particularly cardiovascular disease and cerebrovascular disease, in a mammalian population.
2. A dietary supplement as claimed in claim 1, *characterised in that* the supplement is fortified by addition of an effective amount of each of at least two compounds selected from Group I.
- 730 3. A dietary supplement as claimed in claim 1, *characterised in that* the concentration of folic acid is such that an effective amount (for an adult human) of from about 300 to about 500 micrograms intake per day is made available by consumption of the dietary supplement.
4. A dietary supplement as claimed in claim 1, *characterised in that* the concentration of cobalamin is such that an effective amount of from about 4 to about 7 micrograms intake per day is made available by consumption of the dietary supplement.
- 735 5. A dietary supplement as claimed in claim 1, *characterised in that* the concentration of pyridoxine is such that an effective amount (for an adult human) of from about 1.5 to 4 milligrams intake per day is made available by consumption of the dietary supplement.
6. A dietary supplement as claimed in claim 1, *characterised in that* the concentration of betaine is such that an effective amount (for an adult human) of from about 100 milligrams to 1 gram intake per day is made available by consumption of the dietary supplement.
- 740 7. A method for controlling the incidence of neural tube defects in a population, comprising supply to the population of a dietary supplement as claimed in claim 3.
8. The use, in the manufacture of a dietary supplement, of an effective amount of at least one compound selected from group I together with at least one fraction derived from milk; the dietary supplement, when consumed, being capable of reducing tHcy and thereby of reducing VaD in a population.
- 745 9. A dietary supplement as claimed in claim 1, *characterised in that* the milk of the dietary supplement further has a bovine origin and a beta casein content substantially comprised of

750 the A2 variant, so that the dietary supplement is capable of reducing the incidence and/or effects of VaD in the population, both as a result of reducing tHcy and as a result of reducing the incidence of diabetes.

10.A dietary supplement as claimed in claim 1, ***characterised in that*** the milk of the dietary supplement further has a bovine origin and has a controlled beta casein content for which at
755 least the A1 and the B variants are substantially excluded, so that the dietary supplement is capable of reducing the incidence of VaD in the population, both as a result of reducing tHcy and as an indirect result of reducing the incidence of diabetes.

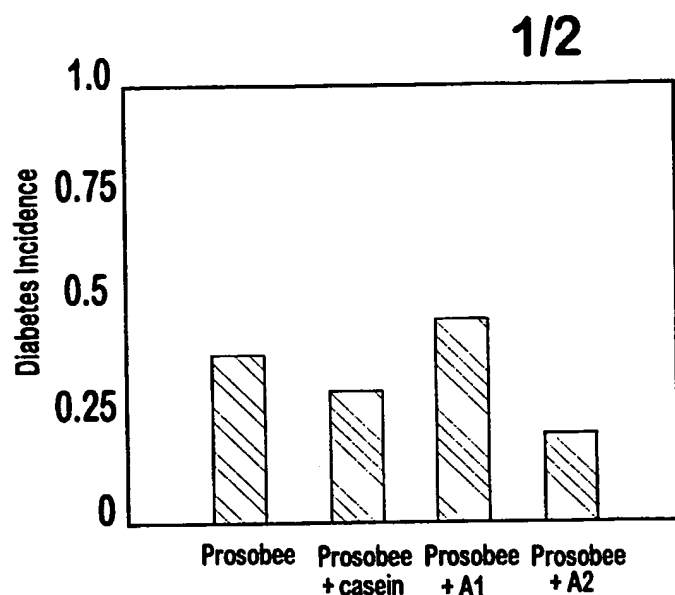
11.A dietary supplement as claimed in claim 9, ***characterised in that*** the milk of the dietary supplement is capable of developing an immunological property during a process of digestion
760 exposing, as a residue of digestion of the A2 beta casein, a relatively stable peptide known as β -casomorphin 9, capable of promoting an immune response within the body.

12.A dietary supplement as claimed in claim 1, ***characterised in that*** a relatively stable active peptide known as β -casomorphin 9 or an analogue thereof is included in the supplement so as to be capable, on ingestion by an individual, of being released into the gut so that the dietary
765 supplement is capable of promoting immunity against diabetes.

13.A dietary supplement as claimed in claim 12, ***characterised in that*** the relatively stable active compound capable of promoting immunity against diabetes is included within a slow-release formulation so that it is released into the gut over a period of time.

14.A dietary supplement as claimed in claim 12, ***characterised in that*** the active compound
770 capable of promoting immunity against diabetes is assisted by the inclusion of at least one agent capable of enhancing a development of immunity within the dietary supplement.

15.A method for reducing the incidence in a population of at least one of: (a) diabetes type I, (b) diabetes type II, (c) cardiovascular disease, (d) cerebrovascular disease, (e) peripheral vascular disease, (f) neural tube defects, or (g) degeneration of blood vessel walls, comprising the steps
775 of manufacturing and providing to the population a dietary supplement in the form of a milk product including A2 beta-casein but substantially no A1 nor B beta-casein, and fortified by addition of an effective amount of at least one compound selected from Group I.

**Fig 1**

H-Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser-Leu-Ser-Ser-Ser-Glu- 20

Glu-Ser-Ile-Thr-Arg-Ile-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Glu-Ser-Glu-Glu-Glu-Glu-Glu-

Thr-Glu-Asp-Glu-Leu-Glu-Asp-Lys-Ile-His-Pro-Phe-Ala-Glu-Thr-Glu-Ser-Leu-Val-Tyr- 60

Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Glu-Asn-Ile-Pro-Pro-Leu-Thr-Glu-Thr-

Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Glu-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu- 100

Ala-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-

Glu-Ser-Glu-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-Asn-Leu-His-Leu-Pro-Pro-Leu-Leu-Leu- 140

Glu-Ser-Trp-Met-His-Glu-Pro-His-Glu-Pro-Leu-Pro-Pro-Thr-Val-Met-Phe-Pro-Pro-Glu-

Ser-Val-Leu-Ser-Leu-Ser-Glu-Ser-Lys-Val-Leu-Pro-Val-Pro-Glu-Lys-Ala-Val-Pro-Tyr- 180

Pro-Glu-Arg-Asp-Met-Pro-Ile-Glu-Ala-Phe-Leu-Leu-Tyr-Glu-Glu-Pro-Val-Leu-Gly-Pro-

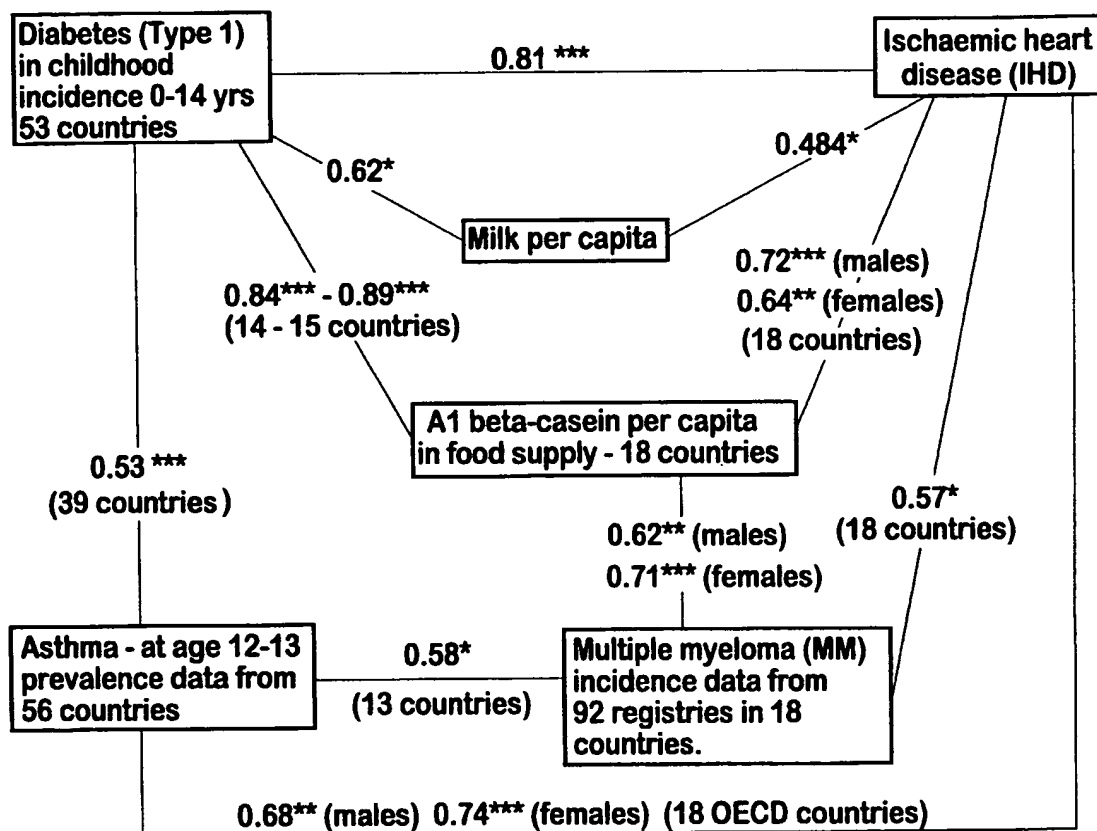
209

Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH

Bovine beta-casein A2.

Fig 3

2/2



* = $p < 0.05$
 ** = $p < 0.01$
 *** = $p < 0.001$

Fig 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00116**A. CLASSIFICATION OF SUBJECT MATTER**Int. Cl. ⁷: A23L 1/305

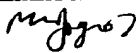
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHEDMinimum documentation searched (classification system followed by classification symbols)
SEE ABOVEDocumentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE BELOWElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN (WPIDS, FSTA and Medline): milk or dairy and betaine/cobalamin/folic/pyridoxine**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/34497 (Shapira, N) 25.09.97 ; see pp7-17	1-15
X	WO 99/03365 (N V Nutricia et al) 28.01.99; see p5 and Table 1; claims 1-7	1-15
X	WO 99/13737 (Shapira, N) 25.03.99; see pp 1-9	1-15

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

- | | |
|---|---|
| <ul style="list-style-type: none">* Special categories of cited documents:"A" document defining the general state of the art which is not considered to be of particular relevance"E" earlier application or patent but published on or after the international filing date"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)"O" document referring to an oral disclosure, use, exhibition or other means"P" document published prior to the international filing date but later than the priority date claimed | <ul style="list-style-type: none">"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art"&" document member of the same patent family |
|---|---|

Date of the actual completion of the international search
25 September 2000Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ00/00116

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 951842 (N V Nutricia et al) 27.10.99; see pp4-6, Table 1	1-15
X	WO 98/19690 (Bristol-Myers Squibb Co) 14.05.98; see pp 3-26, claims	1-15
X	The Australian J of Dairy Technology 52, pp109-118 (1997) Parodi, P. W. "Cow's milk folate binding protein: its role in folate nutrition" ; see pp109-112	1-15
X	Bailliere's Clinical Haematology 8, pp567-601 (1995) Linnell et al "Inherited errors of cobalamin metabolism and their management"	1-4
X	J of American Dietetic Association 97, pp167-173 (1997) Koehler et al "Folate nutrition and older adults: challenges and opportunities"	1-3

International application N .
PCT/NZ00/00116

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9903365	AU	84658/98	EP	891719	EP	1001685
WO	9913737	AU	89960/98	US	5993866		
EP	951842	WO	200042868	WO	200043013		
WO	9819690	AU	52442/98	EP	951293	US	6008221
WO	9734497	AU	18099/97	EP	906025		

END OF ANNEX